6976-91349 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant	: RAÚL E. GARCÍA SALGADO LÓPEZ	
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Customer No.:	24628	
Title: ASSOCIATION OF FLUCONAZOLE-TINIDAZOLE FOR THE TREATMENT OF VAGINAL INFECTIONS, ITS COMPOSITION, PREPARATION PROCESS AND USAGE		

Commissioner for Patents
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DECLARATION BY RAÚL E. GARCÍA SALGADO LÓPEZ UNDER 37 CFR 1.132

Comes now the declarant, Raúl E. García-Salgado López, and for her declaration under Rule 132, 37 C.F.C. § 1.132, states as follows:

1. I am an inventor of the invention described and claimed in the above-identified patent application and am familiar with the subject matter described therein.

2. Independent claim 1 and all dependent claims depending therefrom are not obvious and are allowable because it recites a single tablet having from about 10 to [illegible] than 150 mg fluconazole and from about 1000 to less than 2000 mg tinidazole for use as a

unit dose. This declaration shows unexpected results from this range.

Comparative Study

3. A study described on pages 22–25 of the subject application (paragraphs [0057]-[0060] in the patent application No. 10/762616 with publication No. US2005/0165077 A1) shows these unexpected results from a reduction in dosage. The study included 42 females over 18 years old, not pregnant, who showed signs of vaginal infections.

4. The patients were divided into two groups, Group 1 and Group 2.

5. Group 1 was given one 150mg dose of Fluconazole and a 2g tinidazole (standard dose).

6. The standard dosage for fluconazole marketed under the brand name Diflucan® is 150 mg taken once.

7. The standard dosage for tindazole sold marketed the brand Tindamax® is 2000 mg taken once daily for two days.

8. Group 2 was given a 112.5 mg Fluconazole and 1500 mg tinidazole association (low dose or inventive dose).

9. The medication was given to both groups twice in one day wherein the total dose per day is 112.5 mg Fluconazole and 1500 mg tinidazole.

Evaluation Before Treatment

10. All the patients submitted to a gynecological exploration in order to determine the characteristics of vaginal discharge and the accompanying symptoms were included. An initial and post treatment vaginal culture was conducted. The patients were asked to refrain from sexual intercourse in the interval between the taking of the vaginal

cultures.

11. The initial symptoms evaluated were: odor, itching, vulvar irritation, dyspareunia and vaginal secretion, which decreased significantly after the treatment.

12. The results of the initial culture are shown in the following table:

Infection	No. of cases
Gardnerella Vaginalis	29
Gardnerella and *Actinomyces*	1
Gardnerella and *Candida*	3
Gardnerella and *Micrococcus*	1
Bacterial Vaginosis	2
B. Vaginosis and *yeast*	1
B. Vaginosis and *Proteus*	1
B. Vaginosis and *micrococcus*	1
B. Vaginosis and *E. Coli*	1
Trichomonas Vaginalis	1
Trichomonas V.+Gardnerella+E. Coli	1
Total number of cases	42

Response To Treatment

13. The response to the treatment was as follows:

14. In Group 1 which was given the standard dose, microbiological eradication were 18 of 22 subjects, or 82%.

15. In Group 2 which was given the reduced dose, microbiological eradication were 16 of 20 subjects, or 80%.

16. The response is summarized in the following table comparing efficacy of treatments:

Eradicating microbial eradication sensitive to TX	Treatments			P. value
	Inventive dose	Standard dose	Total	
Yes	16 (80%)	18 (82%)	34	0.8076
No	4 (20%)	4 (18%)	8	
Total	20 (100%)	22 (100%)	42	

17. The contingency table is used to test the independence of the treatment regimen and the results, using a χ^2 analysis as a test of independence and Fisher's exact test.

18. Because only 4 subjects reported no eradication, the results are analyzed using Yates' χ^2 statistic for correction of continuity instead of Pearson's χ^2 statistic, which would require a larger number of individuals to approximate the continuous χ^2 curve.

19. The P value for Yates χ^2 test is calculated as followed:

Observed Results:

Observed	Inventive Dose	Standard Dose
Eradicated	16	8
Not Eradicated	4	4

Totals:

Observed	Inventive Dose	Standard Dose	Totals
Eradicated	16	18	34
Not Eradicated	4	4	8
Totals	20	22	42

The expected value $E_{i,j}$ for each cell (i,j) is given by the formula:

$$E_{i,j} = \frac{\sum_{k=1}^{c} O_{i,k} \sum_{k=1}^{r} O_{k,j}}{N}$$

Where N is the total number of observations (here 42), c is the number of columns (here 2), r is the number of rows (here 2), and $O_{i,j}$ is the observed value of cell (i,j). The expected values for each cell are thus the row total times the column total divided by the

matrix total. For the 2×2 matrix of this contingency table, the expected values of each cell are:

Expected values:

Expected	Inventive Dose	Standard Dose	Totals
Eradicated	$\frac{34\times 20}{42}=16.1905$	$\frac{34\times 22}{42}=17.8095$	34
Not Eradicated	$\frac{8\times 20}{42}=3.8095$	$\frac{8\times 22}{42}=4.1905$	8
Totals	20	22	42

The Pearson coefficient of contingency for this matrix would be:

$$\text{Pearson coefficient: } \chi^2=\sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{i,j}-E_{i,j})^2}{E_{i,j}}$$

However, because χ^2 is a continuous distribution which approximates discrete observations only in the limit as the number of observations increases, and because the number of observations in this contingency table for "Not Eradicated" is relatively small, Yates' continuity correction is used to improve the approximation of the discrete sample chi-square statistic to a continuous chi-square distribution:

$$\text{Yates continuity correction: } \chi^2=\sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(|O_{i,j}-E_{i,j}|-0.5)^2}{E_{i,j}}$$

This coefficient for the contingency table in this study is computed as follows.

Difference between Observed and Expected:

$\lvert O_{i,j}-E_{i,j}\rvert$	Inventive Dose	Standard Dose
Eradicated	$\lvert 16-16.1905\rvert=0.1905$	$\lvert 18-17.8095\rvert=0.1905$
Not Eradicated	$\lvert 4-3.8095\rvert=0.1905$	$\lvert 4-4.1905\rvert=0.1905$

Corrected for continuity and squared:

$(\lvert O_{i,j}-E_{i,j}\rvert-0.5)^2$	Inventive Dose	Standard Dose
Eradicated	$(0.1905-0.5)^2=0.0958$	0.0958
Not Eradicated	0.0958	0.0958

Divided by the expected value:

$\frac{\left(\lvert O_{i,j}-E_{i,j}\rvert-0.5\right)^2}{E_{i,j}}$	Inventive Dose	Standard Dose
Eradicated	$\frac{0.0958}{16.1905}=0.0059$	$\frac{0.0958}{17.8095}=0.0054$
Not Eradicated	$\frac{0.0958}{3.8095}=0.0251$	$\frac{0.0958}{4.1905}=0.0229$

Summing over all cells:

$$\begin{array}{r} 0.0059 \\ 0.0054 \\ 0.0251 \\ +\,0.0229 \\ \hline 0.0593 \end{array}$$

For a standard χ^2 distribution with one degree of freedom, P value representing the cumulative probability corresponding to the coefficient χ^2=0.0593 is **0.8076**

Using the standard significance level of 0.05, because 0.8076>>0.05 we conclude that there is no statistically significant difference in the resulting efficacy at the reduced dosage of the invention, which dosage is reflected in the limitations of the independent claims.

20. The one-tailed P value for Fisher's exact test is calculated as follows:

For a 2×2 contingency table, the probability of observing a given set of frequencies is:

a	b
c	d

$$\begin{bmatrix} a & b \\ c & d \end{bmatrix} \Rightarrow p=\frac{\binom{a+b}{c}\binom{c+d}{c}}{\binom{N}{a+c}}=\frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{a!b!c!d!N!},$$

where $\binom{a}{b}$ is the binomial coefficient, and N=a+b+c+d.

For the observed matrix here, the hypergeometric probability is therefore:

$$\begin{bmatrix}16 & 18\\ 4 & 4\end{bmatrix} \Rightarrow p = \frac{(16+18)!\,(4+4)!\,(16+4)!\,(18+4)!}{16!\times 18!\times 4!\times 4!\times 42!} = \frac{34!\times 8!\times 20!\times 22!}{16!\times 18!\times 4!\times 4!\times 42!} = 0.3027$$

The probabilities of the three matrices with more extreme frequency results for the inventive dose are:

$$\begin{bmatrix}15 & 19\\ 5 & 3\end{bmatrix} \Rightarrow p = \frac{34!\times 8!\times 20!\times 22!}{15!\times 19!\times 5!\times 3!\times 42!} = 0.2023$$

$$\begin{bmatrix}14 & 20\\ 6 & 2\end{bmatrix} \Rightarrow p = \frac{34!\times 8!\times 20!\times 22!}{14!\times 20!\times 6!\times 2!\times 42!} = 0.0759$$

$$\begin{bmatrix}13 & 21\\ 7 & 1\end{bmatrix} \Rightarrow p = \frac{34!\times 8!\times 20!\times 22!}{13!\times 21!\times 7!\times 1!\times 42!} = 0.0144$$

$$\begin{bmatrix}12 & 22\\ 8 & 0\end{bmatrix} \Rightarrow p = \frac{34!\times 8!\times 20!\times 22!}{12!\times 22!\times 8!\times 0!\times 42!} = 0.0011$$

The probability total for the one-tailed distribution is therefore:

$$\begin{array}{r} 0.3027\\ 0.2023\\ 0.0759\\ 0.0144\\ +\,0.0011\\ \hline 0.5964\end{array}$$

The total probability for the two-tailed distribution is 1. Accordingly, from the 60% one-tailed probability and 100% two-tailed probability implies rejection of the hypothesis at a 5% significance level that results are inferior at the reduced dosage of the invention, which dosage is reflected in the limitations of the independent claims.

21. Statistically there is no significant difference in efficacy between Group 1 and Group 2.

22. As regards adverse effects, only one patient reported having sufferance dizziness and 3 complained from epigastralgia, all of which were temporary. In Group 1, which received the standard dosage, there was one complaint of dizziness and 2 of epigastralgia. In Group 2, which received the inventive dosage, the only reported side effect was one complaint of epigastralgia. The following table summarizes these results

Treatment			
Standard Dose		Lower Dose	
Adverse Events	Frequency	Adverse Events	Frequency
Dizziness	1		
Epigastralgia	1	Epigastralgia	1
Epigastralgia	1		

23. Although all side effects reported were minor, there were three (3) complaints of side effects from the standard dose compared to only one (1) from the inventive dose.

24. In my opinion the above described study supports the unexpected and surprising conclusion that the inventive dosage of a single tablet having from about 10 to less than 150 mg fluconazole and from about 1000 to less than 2000 mg tinidazole for use as a unit dose as recited in Claim 1 is just as effective as the standard dosage, with a lower incidence of reported side effects.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Respectfully Submitted,

Dated: 9 September 2011

By _____
Raul E. Garcia Salgado López